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Experimental Pulmonary Sarcoma Metastases in Athymic Nude Mice

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Background: Pulmonary metastases remain a challenging therapeutic problem in the treatment of patients with soft tissue sarcomas. A pulmonary sarcoma metastases animal model might facilitate studying the biology of metastases, diagnosis, and treatment modalities of this disease. Intravenous injection of human tumor cells into nude mice has been reported using human melanoma and colorectal carcinoma to produce pulmonary metastases. Human fibrosarcoma cells were intravenously administered to athymic nude mice to simulate clinical pulmonary metastases.
Methods: HT-1080 human sarcoma cells derived from a poorly differentiated fibrosarcoma were used to prepare inoculant at a concentration of 5×10^6 cells per ml. Male athymic nude mice were injected subcutaneously with 1×10^6 cells in the right hind flank and sacrificed when the tumors were 1–2 cm in diameter. Age- and weight-matched athymic nude mice were intravenously injected through tail veins with 10^4, 10^5, and 10^6 cells. The mice were sacrificed at 7, 14, and 21 days after intravenous injection of the tumor cells. Tissues were histologically examined for pulmonary metastases.
Results: Neither gross nor microscopic spontaneous metastases were found in any of the animals that received subcutaneous xenografts, and no pulmonary metastases were identified in mice intravenously injected with $<10^5$. All mice inoculated with 10^6 cells developed tumor colonies in the lungs, which were microscopically evident as early as day 7. No metastases were found in the liver, spleen, heart, or other tissues. In a second experiment, HT-1080 cells were injected at 10^6; all animals developed lung metastases and died of lung tumor involvement, with an average survival of 35 days.
Conclusions: These experiments identify a sarcoma animal pulmonary metastases model that is readily available, relatively inexpensive, easily utilized, and reproducible.
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KEY WORDS: lung tumor; xenograft; fibrosarcoma

INTRODUCTION

Clinical soft tissue sarcomas commonly disseminate hematogeneously metastasizing to lung. The pulmonic parenchyma is the most frequent metastatic site, involved in 80% of patients found with metastatic sarcoma and as the only site of involvement in 70% [9,17]. Despite im-

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provements in the treatment of local soft tissue sarcomas over the years, pulmonary metastases remain a challenging therapeutic problem, accounting for the death of a significant number of these patients. Therefore, a pulmonary sarcoma metastases animal model might facilitate studies regarding the biology of metastases, diagnosis, and treatment modalities of this disease entity.

Athymic nude mice are readily available and xenografted human tumors grown in these mice maintain their karyotype, histologic appearance, and most biochemical characteristics [15]. Subcutaneous human tumor xenografts in athymic nude mice is a frequently used model for in vivo tumor studies. However, spontaneous metastases rarely occur in athymic mice with subcutaneous tumor xenografts [3]. Intravenous injection of human tumor cells into the nude mice has been previously reported using human melanoma [4,10] and colorectal carcinoma to produce pulmonary metastases. This report depicts a similar technique utilizing i.v. administration of human fibrosarcoma cells into athymic nude mice to simulate clinical pulmonic metastases.

MATERIALS AND METHODS

Animals

Athymic NCr-nu/nu male mice were purchased from the NCI Frederick (MD) Cancer Research Facility. Animals were kept in the rooms maintained at constant room temperature (22–24°C) and humidity (30–50%). Food and bedding were sterilized and animals were given tap water in sterilized bottles. The guidelines of the Hektoen Institute for Medical Research for the care and use of laboratory animals were followed in the performance of this study.

HT-1080 Human Sarcoma Cell Line

HT-1080 is a tumor cell line derived from a poorly differentiated fibrosarcoma of a 35-year-old Caucasian man who died without having received chemotherapy or radiotherapy [13]. These cells are cultured and maintained with Eagle's minimum essential medium (EMEM) with Earle's salts, supplemented with 10% heat-inactivated fetal calf serum (FCS), 1 ml/100 ml of 100× nonessential amino acids, 1 ml/100 ml of 100× glutamine, and 1 ml/100 ml of 100× antibiotic-antimycotic solution (GIBCO, Grand Island, NY). This cell line was obtained from the American Type Culture Collection (Rockville, MD).

Monolayer HT-1080 cells were grown to near confluence, harvested with EDTA, washed three times with phosphate-buffered saline (PBS), and resuspended in EMEM to have a concentration of 5×10^6 cells/ml. The cells were confirmed by trypan blue to have a viability of >95%.

Subcutaneous Xenografts

Male athymic nude mice, 8 weeks old and of 24 g average weight, were injected subcutaneously with 1×10^6 HT-1080 cells in the right hind flank. The animals were sacrificed and autopsied when the tumors attained a 1–2 cm diameter.

Experimental Pulmonary Metastases in Athymic Nude Mice

A pulmonary sarcoma metastatic model was devised using age- and weight-matched athymic NCr-nu/nu nude mice. The mice were anesthetized with intramuscular ketamine HCl (Parke-Davis, Morris Plains, NJ) and injected intravenously (i.v.) through tail veins with various amounts of HT-1080 cells (10^4, 10^5, 10^6). The mice were checked daily and the survival time was recorded. The mice were sacrificed by carbon dioxide asphyxiation 7, 14, and 21 days after intravenous injection of the tumor cells.

Histologic Examination

Tissues from the mice were collected, weighed, and fixed in 2% paraformaldehyde for histological examination to detect pulmonary metastases. The sectioned tissues were stained with hematoxylin and eosin.

RESULTS

Subcutaneous Xenografts

All animals developed tumors of similar size at each injection site. At 10–17 days following injection of HT-1080 cells, the tumors were ~1 cm in diameter in five animals. The tumors were 2 cm in diameter in another five animals 15–24 days after inoculation of tumor cells. Neither gross nor microscopic spontaneous metastases were found in any of these animals. This result correlated with our previous localization studies in this animal tumor model [1,2].

Experimental Pulmonary Metastases

No pulmonary metastases were identified in mice i.v. injected with $<10^5$ HT-1080 cells. Only two of the six (33%) mice injected with 10^5 cells developed pulmonary colonies, one on Day 14 and another on Day 21 after injection of the tumor cells. When an inoculation of 10^6 cells was i.v. injected, all of the 24 mice were found with tumor colonies in the lungs, which were microscopically evident as early as Day 7 (Fig. 1). The number and size of tumor colonies increased in this group with time. Multiple 0.25–0.50 mm metastatic nodules were grossly visible on Day 21. There were no metastatic colonies found in the liver, spleen, heart, or other tissues. A second experiment was performed to determine animal survival following i.v. injection of 10^6 HT-1080 cells. Again, all animals developed lung metastases, and all six animals

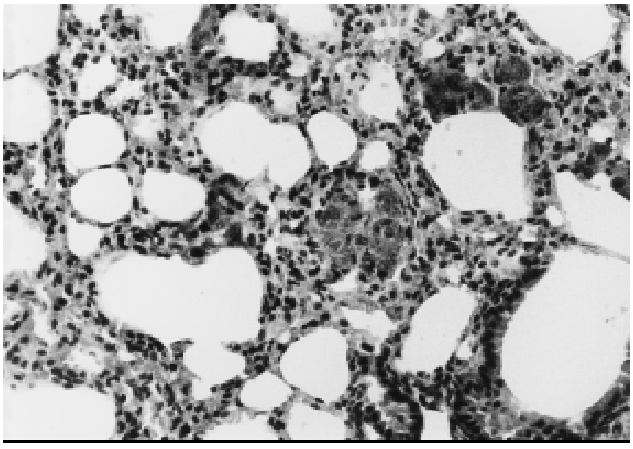

Fig. 1. Distinct, well-defined tumor colonies scattered in the pulmonary parenchyma 7 days after injection of 10^6 human sarcoma cells (HT-1080) through lateral tail veins into athymic nude mice. There were no tumor deposits in the vessels (×100).

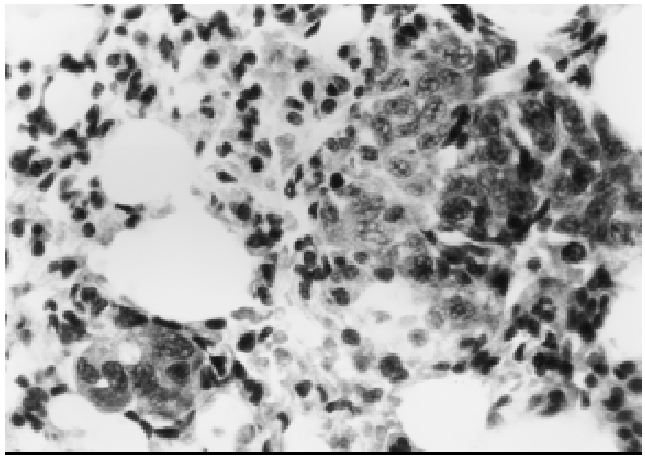

Fig. 2. Tumor colonies 14 days after injection of 10^6 of HT-1080 cells. Confluent tumor colonies were found. Hyperchromatic nuclei were obvious (×400).

died of pulmonic tumor involvement with an average survival of 35 days.

Histology of Tumor Colonies

Pulmonic metastatic tumor deposits were evident 21 days following i.v. injection of 10^6 HT-1080 cells. Various-sized tumor colonies ranging from 0.25 to 0.50 mm were identified scattered over the surface of the lungs as well as in the lung parenchyma (Figs. 1, 2). The tumors also appeared as distinct subpleural nodules (Fig. 3). No tumor cells were found in blood vessels. The size and number of pulmonic tumor colonies increased proportionally with time.

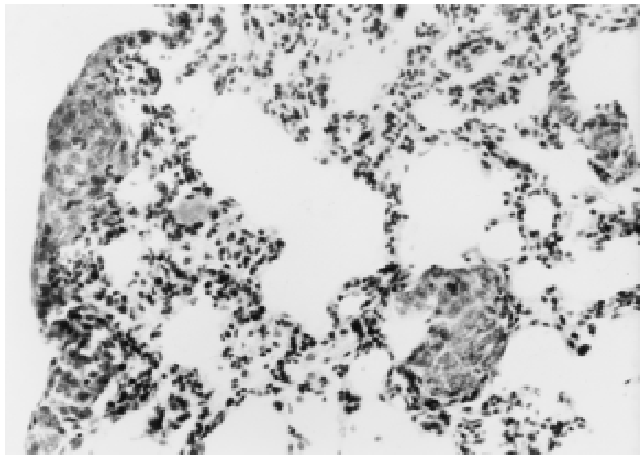

Fig. 3. Tumor colony at subpleural area that was found 14 days after injection of sarcoma cells (×100).

DISCUSSION

Metastasis formation is a complex, multistep process that is determined by both host factors and intrinsic properties of the tumor cells [12]. To establish metastases, the primary tumor cells must invade the surrounding tissues, penetrate into blood vessels and/or lymphatic vessels, survive in the circulation, be arrested in the capillary bed of distant organs, extravasate into organ parenchyma, and proliferate to form metastatic tumor. Experimental metastasis formation, however, often refers to tumor colonies produced after intravenous injection of tumor cells. Although intravenous injection of tumor cells bypasses the initial steps in metastasis formation, all the subsequent steps must occur before such metastases can be established.

As in this and other investigations, spontaneous metastases do not ordinarily occur in adult athymic nude mice having subcutaneous xenografts [14,16]. The absence of metastases formation in athymic mice has been attributed to high natural killer (NK) cell activity in adult nude mice. Furthermore, reports have cited occurrence of tumor metastases in the recipients with low NK cell activity [6,7]. Accordingly, young (<3 weeks), immunologically immature mice with low NK activity have been the animals suggested by the various authors to form spontaneous lung metastases from subcutaneous human tumor xenografts. Pulmonic metastases have been recently reported in adult athymic mice with i.v. injection of human tumor cells [4,5,10]. In this study, i.v. injection of 10^6 human sarcoma cells into adult athymic nude mice produced pulmonic metastases in all recipient animals. This finding substantiates other investigators' findings using various human tumor xenografts in adult mice.

Authors have suggested that the metastatic potential of tumor cells may be related to membrane glycoproteins [11]. Reduction or loss of membrane expression of glycoprotein antigens may occur after in vitro culture of tumor cells, which could alter these cells' metastatic capacity [8,18]. However, Sharkey et al. [5] and Giavazzi et al. [14] have shown that no differences exist in lung colonizing potential in tumor cells taken after serial pas-

sages in nude mice compared to in vitro cultured cells. For this series of experiments, a single cell suspension detached from the in vitro monolayer cultured cell line was utilized to produce lung metastases. This method was selected due to simplicity and on the findings of other investigators who have used this same method successfully to produce pulmonic colonies after i.v. injection of tumor cells [4].

The results of this experiment are encouraging, identifying a sarcoma animal pulmonic metastases model that is readily available, relatively inexpensive, easily utilized, and reproducible. Further experiments utilizing this system are in progress in order better to understand the biology of such tumor deposits to devise novel detection and therapeutic techniques for such metastases in patients.

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COMMENTARY

This paper reports that one needs to inject 10^5 or more sarcoma cells intravenously into athymic mice for pulmonary metastases to develop and therefore cites further evidence on the importance of nonspecific host mechanisms, such as natural killer cells, in eradicating microscopic amounts of tumor. In previous experiments we too concluded that the need to inject 10^3 cells or higher amounts for successful take in a subcutaneous location in syngeneic or allogeneic tumor models did not appear to be related to overcoming any systemic host resistance. The relatively large number of cells to initiate a subcutaneous growth apparently was necessary in order to overcome nonspecific local mechanisms present in the host [1,2].

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